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UNCERTAINTY ANALYSIS OF MULTI RESIDUE METHODS FOR PESTICIDES IN LETTUCE

Pooja Suman[1]*, Pushpendra Kumar Sharma[2] and Dileep K. Singh[3]

[1]Department of Zoology, Miranda House, University of Delhi (Delhi), India
[2]Department of Zoology, D.A.V. (P.G.) College, Dehradun (U.K.), India
[3]Department of Zoology, University of Delhi (Delhi), India

*Corresponding author: pooja.suman@mirandahouse.ac.in

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Abstract: Two methods were developed for uncertainty analysis in lettuce using different processing conditions. Methodology I involved analysis through analytical portion sizes of 5, 15, 50 and 150g which were processed at ambient and low temperature processing conditions. 50 and 150g samples recovered a low percentage (68-72%) of pesticides as compared with 5 and 15g (64-78%) samples. The uncertainty was lowest for 50g samples at ambient temperature and for 15g samples at low temperature processing. 30g analytical portion size was decided to be analyzed through methodology II, resulting in higher recoveries (70-111%) and lower uncertainty (below 11%). The uncertainty and sampling constant determined from internal standard chlorpyrifos was within limit and the pesticide was stable during analysis. The processing of samples at low temperature (i.e., cryogenic milling) was more efficient for the analysis of pesticide residues.

Keywords: Chlorpyrifos, Lettuce, Multi residue, Pesticides, Sampling constant, Uncertainty.

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INTRODUCTION

The use of pesticides and its residues on lettuce are of particular food safety interest. The Food and Drug Administration (FDA) found detectable pesticide residues in 55 percent of domestically produced lettuce samples and two percent contained residues exceeding tolerance levels. These residues were analyzed (Rissato *et al.*, 2005) through multi residue methods (MRMs) and these methods were validated to minimize the uncertainty factors (Fajgeli and Ambrus, 2000a, 2000b).

Uncertainty is defined as the systematic error in the multi-residue methods that influences the reproducibility and the repeatability of the analysis (Cuadros-Rodrıguez *et al.*, 2002) and it is being recognized as a probabilistic estimation of the maximum error of a measurement. Three different approaches have been proposed as bottom-up (Fajgeli and Ambrus, 2000c; ISO/IEC 98-3, 2008), top- down (Alder *et al.*, 2001) and in-house validation (AMC, 1995; Thompson *et al.*, 2002) for the expression of uncertainty from the analytical method.

The uncertainty in multi-residue analysis is mainly due to the loss of pesticides during various analytical steps. The extent of the loss is dependent on both the pesticide and the sample

type and probably varies between different varieties and between different samples of the same variety. The losses of pesticides at the sample processing stage and/or subsequent analytical steps will result in an underestimate of residue levels with possible implications on MRL compliance monitoring, consumer risk assessments, and measurement uncertainty. It is therefore desirable to develop and adopt analytical procedures that minimize pesticide losses and improve the reliability of results.

Most laboratories comminute laboratory samples at ambient temperature even though losses for a number of pesticides including chlorothalonil, captan, folpet, and tolylfluanid have been reported to occur during this procedure (Hill *et al.*, 1999; Lyn *et al.*, 2003). There is evidence to suggest that sample processing at low temperature (cryogenic milling) can minimize the extent of the reported losses and thus produce more reliable results procedure (Fajgeli and Ambrus, 2000d).

Uncertainty associated with various analytical steps has been estimated through different approaches (Silva *et al.*, 2000). Relatively few studies have been reported in the literature. Ambrus *et al.* (1996) nicely proposed the use of sampling constants for estimating the uncertainty associated with the ambient sample processing of apples and head cabbages containing incurred pesticide residues. According to the theory of sampling the average residue in a sample taken randomly from an object was an unbiased estimate of the mean residue being in that object. The uncertainty of the analytical results (SR) comprises the uncertainties of sampling (SS), sample preparation (SP) and sample analysis (SA). The standard deviations of residues in replicate samples give the estimate of standard deviation of the sample, which is the measure of the uncertainty of sampling (Ambrus, 1996).

Cuadros-Rodriyguez *et al.* (2002) applied the 'bottom-up' and 'in-house validation' approach in estimation of uncertainty associated with determination of organophosphorus and organochlorine pesticides contained in cucumber. Repeatability of determination of analytes in spiked samples and also uncertainty associated with the preparation of the calibration standard solutions (weighing, diluting) were identified as the most significant sources of combined uncertainty. It was reported that uncertainty value is dependent upon on the concentration of analyte in examined sample. In another study (Ambrus, 1996), significant sources of uncertainty were identified on the basis of statistical comparison (F-test) between (i) combined uncertainty associated with gravimetric, volumetric and chromatographic quantification steps of analytical method and (ii) experimental dispersion of replicated analysis of spiked samples. Stepan *et al.* (2004) studied the critical assessment of both 'bottom-up' and 'top-down' approaches approved for estimation of combined uncertainty of measurement with analysis of pesticide residues in apples. Christensen *et al.* (2003) estimated the uncertainty on the basis of data from in-house validation, it was investigated that there was no difference in relative standard reproducibility (RSDR) between the spiking levels within the single commodities but significant difference was observed in RSDR between the matrices (Štepán *et al.*, 2004).

Using a top-down approach, Lyn *et al.* (2003) concluded that the standard measurement uncertainty associated with physical sample preparation can be high and dominate the overall measurement uncertainty for some pesticide-commodity combinations. Silva *et al.* (2003) proposed a differential method for the estimation of sample processing and sub-sampling performance, based on a comparison of the dispersion of results for the global method with the uncertainty estimated from developed models for the individual analytical steps (bottom-up approach).

The aim of this study was to assess two separate methodologies involving different processing conditions on measurement of uncertainty in multi-residue analysis on lettuce. Uncertainty of sample processing, analytical portion size, uncertainty in analysis, stability of pesticide residues and effect of matrix on pesticide residues are the factors taken into account.

MATERIALS AND METHODS

Chemicals and Reagents

Pesticide reference standards, all 95% or higher purity, were obtained from the Dr. Ehrenstorfer GmbH (Augsburg, Germany). The treating

solutions of pesticides were prepared in ethlyacetate.

Ethyl acetate, Acetone, Cyclohexane and n-Hexane were high purity grade (GR) solvents from Merck. Sodium hydrogen carbonate ($NaHCO_3$), Sodium sulphate (Na_2SO_4), Sodium chloride (NaCl) was also from Merk. Polystyrene: 2% divinylbenzene copolymer beads (200-400 mesh) was from Acros Organics. c-Florisil (60-100 mesh) was from CDH and Dry ice was bought from Laser Gases (Delhi, India).

Equipments and Apparatus

Laboratory chopper, Warring blender (Khera Lab Instruments, Delhi, India), Grinder (Tefon Appliances (India) Private Limited) Homogenizer (Khera Lab Instruments, Delhi, India), Refrigerated Centrifuge (Sorvall) were used to prepare sample for the extraction of pesticide residues.

Methodology

Two different methodologies were used for validation and the uncertainty estimation in the analysis of residues of pesticides spiked on the Lettuce at laboratory conditions. An internal standard, chlorpyrifos (known to be stable under conditions employed), was included in the spiking standard to estimate the stability of pesticides residues and also to measure the sampling constant that determines the efficiency sample processing equipment (Fussell *et al.*, 2002; Bettencourt de Silva *et al.*, 2003; Silva *et al.*, 2003). The chlorpyrifos, a broad spectrum organophosphrous pesticide is normally used against pod borers, fruit borers, stem borers, leaf miners, defoliating caterpillars, sucking pests, termites etc. and in other settings, to kill a number of pests, including insects and worms (Prakash and Verma, 2014).

Methodology- I

The methodology- I involve the spiking of mixture of pesticides on the two batches of 1.5 kg lettuce sample, after the half an hour interval, double step processing was carried out for the whole sample which included the blending and grinding at two different temperatures i.e., ambient and low (by dry ice) temperature. The same procedure was carried for the control samples (two batches of 1.5 kg) without the spiking of pesticide mixture.

Sample processing under ambient condition

The treated samples were blended 6-7 minutes at intervals of 1 minute. The matrix was stirred constantly in between the subsequent intervals. The consistency of the matrix was examined visually by taking the peel size. The peel size was taken by withdrawing 2g of sample directly from the warring blender and diluted it in 1L water, stirred it and allowed the peels to migrate to the surface. Then transferred about 400g of blended matrix to the grinder and then grinded for about 3 minutes to get completely homogenized pulp.

Sample processing with dry ice

Same procedure was carried out when the sample processing was done with dry ice. In this procedure the dry ice was added respectively to the blender and grinder until a free flowing matrix was obtained.

Sampling of the Analytical portions

Three replicates each of 15g and 150g analytical portion were sampled out from the warring blender in 250 mL centrifuge tubes and thick-walled, 1L extraction beakers respectively. Similarly, three replicates each of 5g and 50g analytical portion were taken from the grinder in 50 ml centrifuge tubes and thick-walled, 500 mL extraction beakers respectively.

Extraction

Sodium bicarbonate ($NaHCO_3$) was added to each analytical portions in the ratio of 6:1 (Analytical portion: $NaHCO_3$) i.e. 25g, 2.5g, 8.33g, and 0.83g of $NaHCO_3$ to 150g, 50g, 15g and 5g analytical portion respectively. Warmed up the analytical portion up to 27^0C over water bath and stirred it regularly to avoid overheating.

Then added weighed amount of ethyl acetate in the ratio of 2:1 (i.e. 300ml ethyl acetate to 150g analytical portion). Further Sodium sulphate was added in the ratio of 1:1 w/w analytical portion (i.e. 150±0.1g for 150g analytical portion). Then each analytical portion was homogenized for 1-1.5 minutes. After homogenization, the 5g and 15g analytical portions were centrifuged for 10 minutes at the speed of 2500 rpm. No centrifugation was done for 50 g and 150g analytical portion. These larger analytical portions were just kept aside undisturbed for about 30-45 minutes. The extracts were cleaned up through column was packed 8g of activated

bio beads and eluted through solvent system of Cyclohexane and Ethyl acetate (in 1:1 ratio).

Methodology- II

Methodology- II involved the single step processing of four batches of 300g sample each for under ambient and low temperature conditions. Three batches of sample were spiked with treating solution and forth one was considered as control under both the processing conditions.

Sample processing under ambient condition

Spiked and control samples were grinded in the processor. The matrix was stirred constantly in between the subsequent intervals of grinding.

Cryogenic processing

Both the control and spiked samples were sealed in the polythene bags and placed at -20^{0}C for overnight (16-18 h). Next day samples were grinded along with addition of 300g of dry ice (in parts of 100g). The grinded samples were again placed in plastic bags (half sealed) under -20^{0}C for overnight to allow the carbon dioxide to dissipate.

Extraction

Three replicates of 30g were sub sampled from the processed matrix batches of both the ambient and dry ice experiment. 5g sodium bicarbonate was added to each sample proceeded with the addition of 60ml acetone and 30g of sodium sulphate. Samples were stirred and extracted with homogenizer for 1 minute. After the sedimentation of matrix for 5 minutes, the samples were filtered and again re-extracted for two times with 60ml of acetone. Samples were partitioned with ethyl acetate and were cleaned up through column packed with 20g of activated Florisil by using solvent system of n-hexane and ethyl acetate (in 7:3 ratios).

Preparation of Calibration Solutions

Both solvent and matrix match calibration standards were prepared for the analysis of recovery of pesticides. The five point calibration curves were constructed, over the range 0.165μg ml^{-1} to 1.665μg ml^{-1} for methodology-I samples on the ECD detector. Methodology II samples were analysed through four point calibration curve over the range of 1.25μg ml^{-1} to 25μg ml^{-1} for group I and 12.5μg ml^{-1} to 250μg ml^{-1} for group II pesticides on the ECD detector. The three point calibration curves were constructed for group I and II pesticides on the FTD detector over the range of 2.5μg ml^{-1} to 5μg ml^{-1} and 25μg ml^{-1} to 50μg ml^{-1} respectively.

Gas Chromatography/ Recovery analysis

Gas Chromatography was performed on GC-Shimadzu 17AAF, with HP-1 Column (100% Polydimethylsiloxane 30m × 0.25mm inner diameter 2.65 film thickness). The following conditions were used for the analysis of pesticides spiked in methodology I : N_2 constant flow 1 mL/min, inlet temperature 250^{0}C, injection volume 2µL (split in 1:20), Electron Capture Detector (^{63}Ni) temperature 300^{0}C, temperature program 180^{o}C for 2 min; then 5^{o}C/min ramp to 290^{o}C (3 min). Total run time was 25 min. Methodology II samples were analyzed for group C pesticide residues in following conditions: N_2 constant flow 1mL/min, inlet temperature 250^{0}C, injection volume 2µL (split in 1:20), Electron Capture Detector (^{63}Ni) temperature 300^{0}C, temperature program 180^{o}C for 1min; then 5^{o}C/min ramp to 260^{o}C (1 min) and then 5^{o}C/min ramp to 290^{o}C (1 min). Total run time was 25 min. Group D pesticide residues in methodology II samples were determined in the following conditions: N_2 and make up Gases: Hydrogen and Zero air with constant flow 1mL/min, inlet temperature 250^{0}C, injection volume 2µL (split in 1:20), Flame Thermo-ionic Detector temperature 300^{o}C, temperature program 80^{o}C for 1.5min; then 20^{o}C/min ramp to 180^{o}C; and 5^{o}C/min ramp to 260^{o}C (1 min). Total run time was 25 min.

In the present work Agilent GC-MS was used to confirm the stability of chlorpyrifos. The samples of ambient and dry ice experiment from the methodology-II were changed into acetone solvent for analysis. The scan mode was performed to check the mass of the each peak in the sample to confirm whether any metabolite peak was present are not. The molecular mass of chlorpyrifos was 350.6, its retention time was 8.3 min and main quantitation ions were 97, 197, 258, 286 and 314. The following conditions were used for the confirmation of stability of chlorpyrifos from methodology II sample: Detector-MS interface, EI mode scanning from 45-450 amu, MS Source Temperature: 230^{o}C MS Quadrupole Temperature: 150^{o}C Capillary column: DB-5 MS (0.25mm × 30m × 0.25μ film thickness) Injection Port Temperature: 250^{o}C,

Table 1: Uncertainty and sampling constants calculated from the chlorpyrifos (internal standard) recovery of all replicates of each analytical portion from Methodology I and II ambient experiment.

Analytical portions (g)	Average Recovery %	CVA%	CVSP%	CVL%	Ks
5	68.04	1.19	2.02	1.39	0.002
15	66.82	0.45	2.53	0.54	0.010
50	65.65	0.23	0.75	0.33	0.003
150	64.88	0.16	0.48	0.22	0.003
30	98.99	1.25	1.30	1.80	0.005

Table 2: Uncertainty and sampling constants calculated from the chlorpyrifos (internal standard) recovery of all replicates of each analytical portion from Methodology I and II low temperature experiment.

Analytical portions (g)	Average Recovery %	CVA%	CVSP%	CVL%	Ks
5	72.22	4.42	6.82	5.24	0.023
15	68.92	0.45	1.99	0.63	0.006
50	67.91	0.72	2.03	1.01	0.021
150	67.17	0.51	1.89	0.70	0.053
30	101.72	1.23	1.66	2.06	0.008

Column Temperature Programming: 180°C (2 min), 5°C/min to 290°C (3 min).

RESULTS AND DISCUSSION

Linearity

The linearity was studied by using solvent and matrix match calibration standards. The calibration curves obtained were linear over the entire range studied, with the correlation coefficients higher than 0.99 for each analyte from both the calibration curves. The analysis of methodology-II samples was also studied by solvent and matrix match calibration standards. The correlation coefficients were higher than 0.99 for each analyte.

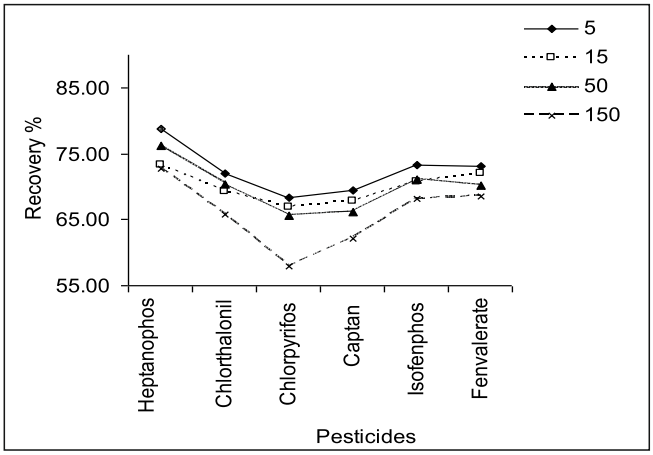

Fig. 1: Graph representing comparison between the average recovery percent of pesticides in different analytical portions from lettuce I ambient experiment.

Efficiency of sample processing

The efficiency of sample processing was characterized by estimating the sampling constant and the uncertainty of sample processing, within tested analytical portion size (Tables 1&2). The sampling constant was calculated as $Ks = W*CV_{SP}^2$, where uncertainty of sample processing (CV_{SP}) was determined from the recovery of chlorpyrifos (I.S) from the analytical portions analyzed (Maestroni *et al.*, 2003).

Recovery and Matrix effect study

The recoveries of each pesticide from the sample

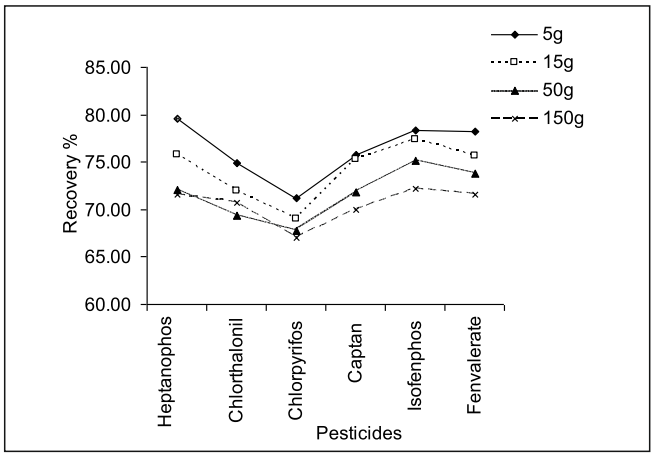

Fig. 2: Graph representing comparison between the average recovery percent of pesticides in different analytical portions from lettuce I dry ice experiment.

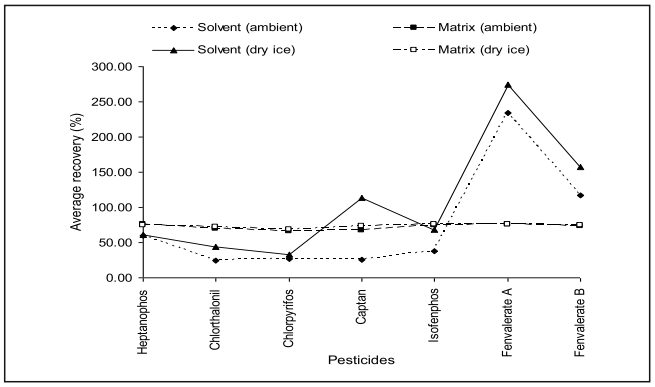

Fig 3: Graph representing the average recovery of pesticides from solvent and matrix match calibrations of the Methodology I ambient and low temperature samples.

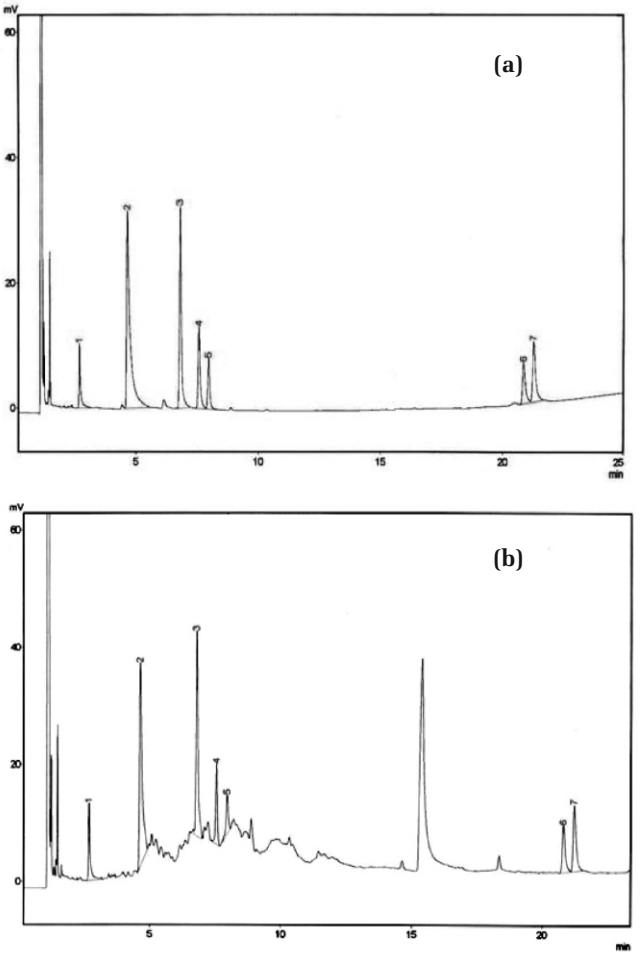

Fig. 5: The GC chromatogram representing (a) Standards in solvent, (b) Standards in matrix of Lettuce detected on ECD from methodology-I (1-Heptenophos, 2-Chlorthalonil, 3-Chlorpyrifos, 4-Captan, 5-Isofenphos, 6 &7-Fenvalerate).

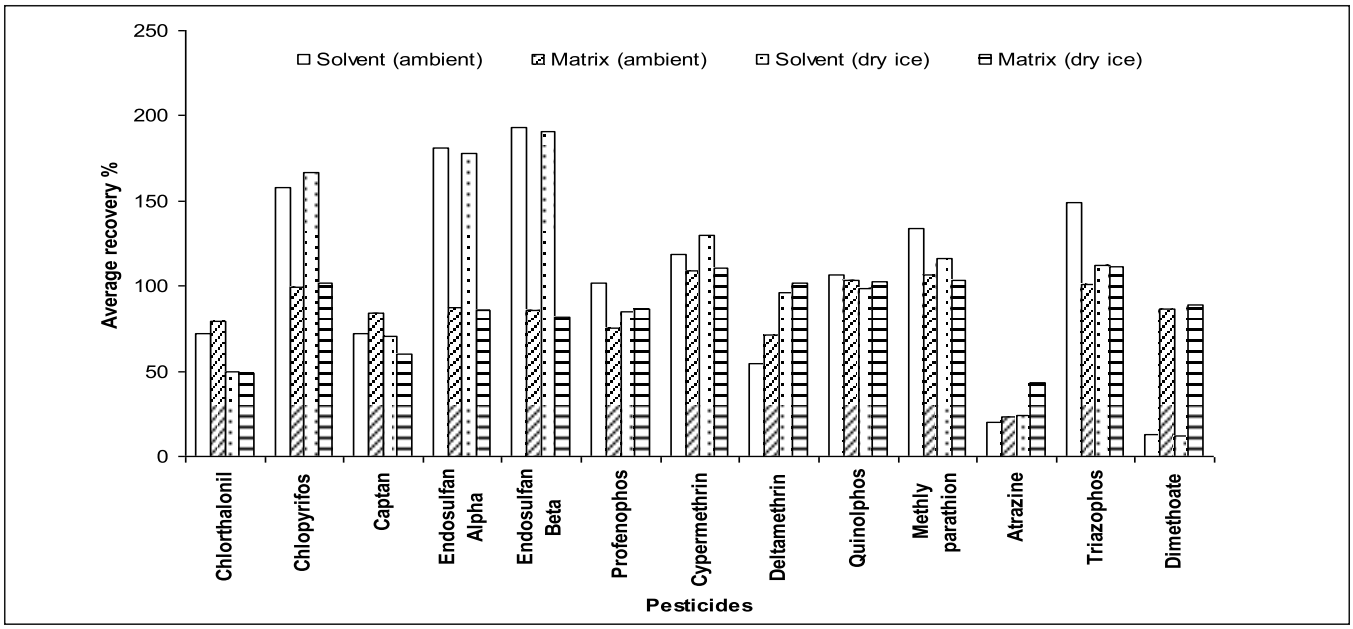

Fig. 4: Graph representing the average recovery of pesticides from solvent and matrix match calibrations of the Methodology II ambient and low temperature samples.

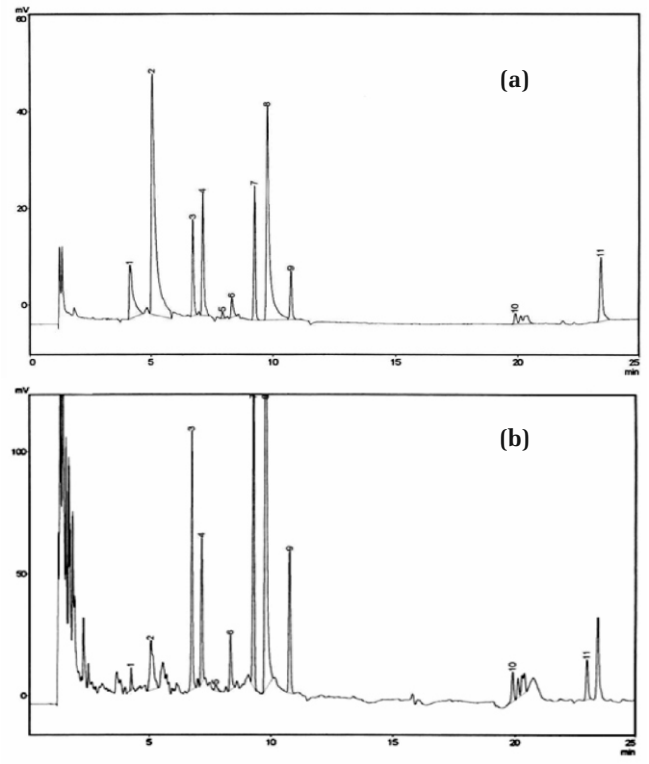

Fig. 6: The GC chromatogram representing (a) Standards in solvent, (b) Standards in matrix of Lettuce detected on ECD from methodology-II (1-Atrazine, 2-Chlorthalonil, 3- Methyl parathion, 4-Chlorpyrifos, 5-Captan, 6-Quinalphos, 7-Profenophos, 8-Endosulfan alpha, 9-Endosulfan beta, 10-Cypermethrin, 11-Deltamethrin.

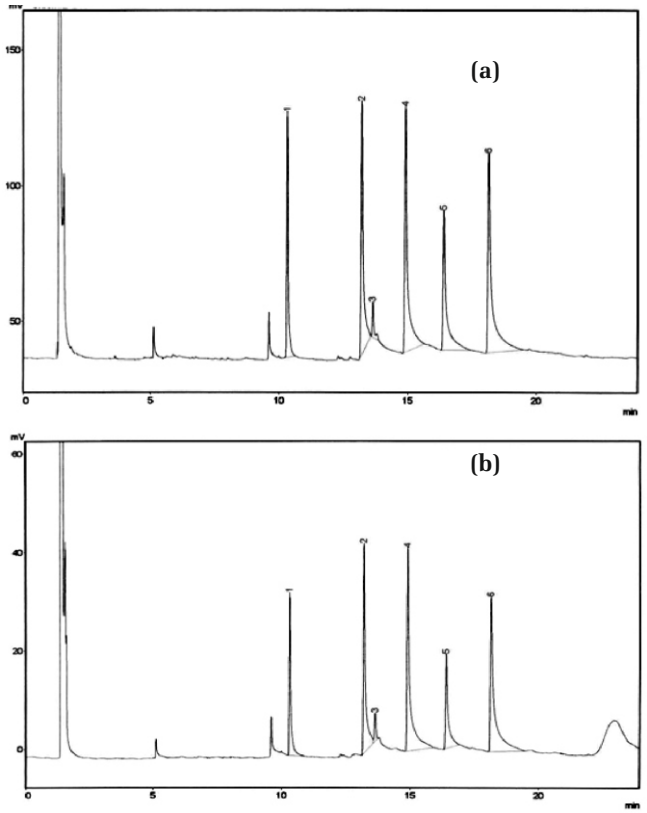

Fig. 7: The GC chromatogram representing (a) Standards in solvent, (b) Standards in matrix of Lettuce detected on FTD from methodology-II (1-Dimethoate, 2-Methyl parathion, 3-Chlorpyrifos, 4-Quinalphos, 5-Profenophos, 6-Triazophos).

at both the temperature conditions were estimated for each replicate of sub sampled analytical portions. The recovery was calculated from the regression equations obtained from both the solvent (SC) and matrix match calibration curves (MC) for all the samples of both the methodologies.

The samples from methodology-I recovered pesticides between 65-75% (Fig. 1&2) at both the temperature conditions. There was decrease in percentage of recovery as the decrease in size of analytical portion, 5g samples recovered pesticides higher as compared to other analytical portions at both the processing conditions. The methodology-II samples resulted in higher recoveries (Fig. 5&6) between the 70-111%. Major pesticides recovered highest at low temperature processing except the recovery of captan and chlorthalonil decreased at same condition (Fig. 4). The same pesticides recovered better at low temperature processing from methodology I. There was also the loss of atrazine

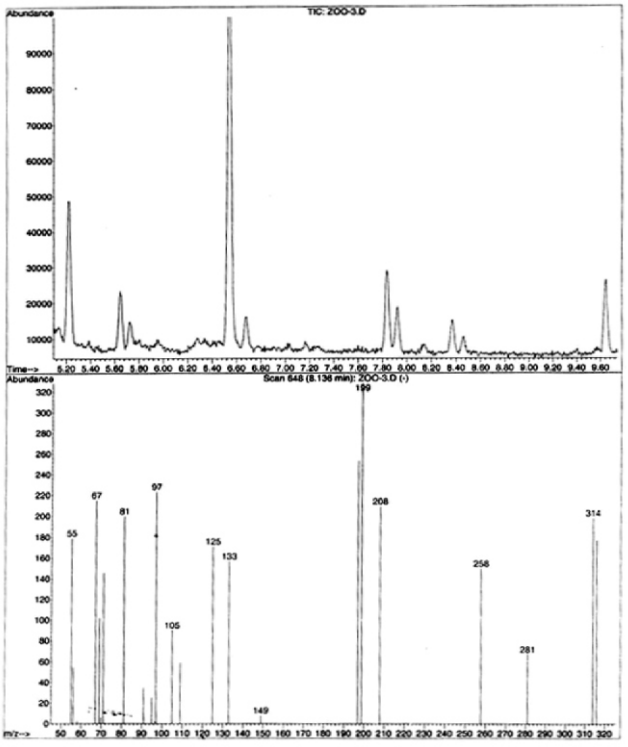

Fig. 8: The GC-MS chromatogram representing analysis of chlorpyrifos stability from Lettuce.

Table 3: Average recovery and uncertainty for pesticides analyzed through methodology-I at ambient temperature processing.

Pesticide	Average Recovery %	CVA%	CVSP%	Cv_{total}%
Chlorthalonil	69.36	2.33	3.25	4.00
Chlopyrifos	66.38	2.11	1.80	2.77
Captan	67.29	2.68	1.84	3.25
Heptenophos	75.29	2.93	3.01	4.20
Isofenphos	73.91	2.41	3.69	4.41
Fenvalarate	74.39	1.79	2.37	2.97

Table 4: Average recovery and uncertainty for pesticides analyzed through methodology-I at low temperature processing.

Pesticide	Average Recovery%	CVA%	CVSP%	Cv_{total}%
Chlorthalonil	71.74	3.51	2.36	4.23
Chlopyrifos	66.75	5.68	-	5.46
Captan	73.19	2.83	3.14	4.23
Heptenophos	74.72	3.04	4.19	5.18
Isofenphos	75.77	2.09	3.02	3.67
Fenvalarate	74.80	1.05	3.41	3.57

Table 5: Average recovery and uncertainty for pesticides analyzed through methodology-II at ambient temperature processing.

Pesticide	Average Recovery %	CVA%	CVSP%	Cvtotal%
Chlorthalonil	79.24	7.06	-	6.38
Chlorpyrifos	98.99	1.25	0.96	1.58
Captan	84.52	6.72	8.55	10.88
Endosulfan	85.56	8.45	-	7.84
Profenophos	75.52	6.47	-	6.23
Cypermethrin	108.67	14.43	11.24	18.29
Deltamethrin	71.63	12.74	4.19	13.41
Quinolphos	103.60	0.17	8.26	19.13
Methly parathion	106.20	0.03	4.95	5.90
Atrazine	23.50	20.73	-	20.28
Triazophos	101.30	5.28	2.84	6.00
Dimethoate	86.56	5.90	7.90	9.86

at both temperature processing conditions causing the lowest percentage recoveries from methodology II.

The matrix effect was studied for both the methodologies and the comparison between the recoveries from the solvent and matrix match

Table 6: Average recovery and uncertainty for pesticides analyzed through methodology-II at low temperature processing.

Pesticide	Average Recovery %	CVA%	CVSP%	Cv_{total}%
Chlorthalonil	48.92	30.50	-	20.48
Chlorpyrifos	101.72	1.23	1.59	2.01
Captan	59.92	2.73	2.19	3.50
Endosulfan	86.11	6.55	3.10	7.24
Profenophos	86.55	2.62	12.02	12.30
Cypermethrin	110.70	4.49	5.91	7.42
Deltamethrin	101.69	11.76	22.02	24.96
Quinolphos	102.92	2.50	3.94	4.67
Methly parathion	104.21	5.07	-	4.50
Atrazine	43.60	10.18	-	9.94
Triazophos	111.05	7.52	3.53	8.30
Dimethoate	88.97	7.61	-	7.48

calibration curves are shown in the figures 3&4. The lettuce matrix resulted both in diminishing and enhancement effect on the recoveries of pesticides. At ambient temperature processing matrix caused diminishing effect on all the analytes except on fenvalerate from the methodology-I samples. Similar effect was studied at low temperature processing; inversely matrix resulted in enhancement effect on captan. A comparison chromatogram shown in figure 5 indicates that the co-extracts of blank matrix interferes the peaks of pesticides (5-10 min of retention time).

The matrix effected recovery of all the analytes of methodology-II and it caused both the type of effects at both the temperature conditions (Fig. 4). The recoveries of chlorpyrifos, chlorthalonil and captan were affected by matrix very similar to the methodology I. The diminishing effect reduced the recoveries of above pesticides at ambient temperature conditions and matrix caused enhancement effect on captan at low temperature conditions. Major enhancement effect was studied on the recovery of endosulfan; it recovered around 200% from SC curve and reduced to 86% from the MC curve at both the temperature conditions. The diminishing effect was also observed on the recoveries of deltamethrin, quinolphos (Fig. 6&7) are the chromatograms showing interferences of matrix on the peak of pesticides detected on ECD and FTD detectors.

The atrazine behaved differently at both temperature conditions, it recovered 149% from SC curve and reduced to 23 % from MC curve at ambient temperature processing. The sample from low temperature condition showed diminishing effect on atrazine and increased recovery from 24% to 44% on correction.

The stability of chlorpyrifos was checked by analyzing the final samples on GC-MS. There was no peak other than of chlorpyrifos which confirmed that the pesticides remained stable during the various processing and analytical steps (Fig. 8).

Uncertainty

The overall uncertainty was calculated for each pesticide between the different batches for both the methodologies (Tables 3 to 6). For methodology I reproducibility of analysis (CV_A), uncertainty of sample processing (CV_{SP}) and total uncertainty (CV_T) were below 6% at both the processing conditions. The lower percentage of uncertainties indicated that the analysis of pesticides was more efficient to produce reproducible results.

The average recoveries of pesticides from the methodology II were deviated from ±0.8 to ±13.4 and quinalphos was the pesticide that deviated highest among the three batches at ambient temperature conditions. The uncertainty of analysis was below 9% for the pesticides except cypermethrin, deltamethrin and atrazine that resulted in CVA above 11%. The atrazine recovered low and resulted in 20% of total uncertainty. The uncertainty of sample processing was below 9% and these values signified that the variations during the sample processing at ambient temperature were within limit. The uncertainty of analysis and total uncertainty for methodology II at cryogenic milling was below 11%, except for chlorthalonil which recovered lower with uncertainty above 20%. The sample processing uncertainty was below 6% except for profenophos (12%). The processing at low temperature conditions resulted in low uncertainty factors on comparison with ambient temperature samples.

CONCLUSIONS

During methodology-I different sized analytical portions that is 5, 15, 50, 150g were analyzed to estimate the precise size of the sample that to be used for pesticide residue analysis. The 5 and 50g analytical portions sub-sampled from second grinding recovered the pesticides efficiently as compared to 15 and 150g analytical portions from first grinding. The overall results concluded that the small sized analytical portions resulted in better recoveries as compared to large sized analytical portions but the high variations were also noticed from small sized analytical portions. Therefore, 30g analytical portion size was decided to be analyzed in the methodology-II.

Methodology-II resulted in better recoveries of all analytes but the uncertainty in the sample processing and analysis at both the processing conditions were on higher side for some pesticides. The atrazine pesticide was the one that was not recovered efficiently from the samples which might be because of its loss during processing at both the temperatures. It was observed that pesticides behaved differently under different processing conditions spiked on same sample and also when analyzed through separate methodologies. The matrix effect was also observed on the recoveries of pesticides on both the methodologies that resulted in increase of uncertainty of analysis. The matrix matched calibrations were suitable and efficient procedure for the correction of matrix effect.

Multi-residue methods are dependent upon type of matrix used for analysis, properties of the pesticides, processing conditions of samples, type of extraction solvent and other analytical steps. In the present work methodology-I was better at both the processing conditions but recovered lower percentage of pesticides. The Methodology-II resulted in efficient recoveries with low uncertainty. All the uncertainty factors were within limit; therefore the analysis performed was reproducible at laboratory conditions. Methodology-II could be used to analyze pesticide residues on food and other matrices to estimate the various uncertainty and variability factors along with the study of effect of the matrix on stability and recovery of pesticides.

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